183 Inhibition of lipopolysaccharide signalling by LL-37 and repercussions for LL-37 function in the *Pseudomonas*-infected cystic fibrosis lung

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Human cathelicidin, LL-37, a 37 amino acid antimicrobial peptide produced by neutrophils and respiratory epithelium, has been shown to possess immunomodulatory properties. Mechanisms of action to explain LL-37's anti-inflammatory activity continue to be elucidated, with a number of publications suggesting potential intracellular targets in addition to extracellular lipopolysaccharide (LPS) and lipotechoic acid (LTA) binding and neutralisation. Therefore, we investigated the effect of LL-37 on LPS signalling pathways in human monocytes. In response to LPS stimulation pre-treatment of monocytic cells with LL-37 inhibited NF-κB activation, STAT-1 and AP-1 signalling pathways. Washing of cells to remove LL-37 abolished any subsequent effects on LPS signalling indicating that LL-37's effects on LPS signalling were mediated extracellularly most likely via direct neutralisation of LPS. We also examined the status of LL-37 in sputa from patients with cystic fibrosis (CF). LL-37 levels were found to be higher in uninfected in comparison to *Pseudomonas aeruginosa*-infected sputa, contrary to expected results, but explained by the significantly higher levels of glycosaminoglycans present in infected samples compared to uninfected. Treatment of these samples with a combination of Dornase alpha and Lyases proved effective in liberating bound LL-37 in infected samples only whilst uninfected samples remained unchanged. Our results suggest that the main mechanism by which LL-37 exerts its immunomodulatory effects on LPS-induced cytokine production is by binding directly to LPS a process which is diminished during *Pseudomonas* infection in CF lung.

184 Plasma chitotriosidase activity in cystic fibrosis patients

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Human chitotriosidase (Chit) is a member of the chitinose family, mainly secreted by activated macrophages and, to a lesser degree, by neutrophils. Chit is markedly increased in pts with lysosomal storage diseases, and, even if to a lesser extent, in other diseases in which an abnormal infiammatory pattern is described.

In this study we investigated whether Chit is augmented in the plasma of CF pts and if it can be a useful marker for inflammatory status in the disease. Serum Chit activity determination was performed, according to Hollak (1994), in 46 CF pts (18 females, mean age 21 yrs, range 1–48, mean FEV1 64% pr, range 22–129). Mean chitotriosidase value was 54.45 nmol/ml/h ±38, higher than the mean value in healthy control group (25.75±17). As in the general population in the 6% of CF pts Chit dosage showed no activity. Chit activity showed no statistical difference in 13 pts with elevated PCR versus 18 pts with normal PCR (64.87, 17.6–209.6 and 42.5, 10.4–104.8 respectively), in the dosage pre- and post e.v. treatment in 11pts (72.5, 20–100 and 52.36, 25–101 respectively) and in 14 pts with normal PCR and FEV1⩾50%pr versus 10 pts with normal PCR and FEV1 < 50% (48.14, 12–104.8 versus 52.32, 1.6–101.6 respectively). Chit activity in 8 pts younger than 15 yrs with normal PCR resulted lower, even if not significantly, than in 6 pts older than 15 yrs with normal PCR (34.45, 10.4–75.6 and 57.71, 25.6–101.6 respectively. $p = 0.087$).

In conclusion Chit activity in CF resulted slighly increased than in nomal controls. It seems to have a better correlation with the age of the patient than with the degree of the BPN and with the presence of a acute exacerbation.

185 Characterization of piperacillin-specific T-cell clones from hypersensitive patients with cystic fibrosis

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Piperacillin (PIP) exposure in patients with cystic fibrosis is associated with a high incidence of delayed-type hypersensitivity reactions. In this study, the nature of the PIP-specific T-cell response was delineated by means of *in vitro* stimulation of lymphocytes and T-cell clones. The four patients each developed maculopapular exanthema and fever (+/– arthralgia or flu-like symptoms) following PIP exposure. Lymphocytes were isolated and incubated with piperacillin for 6 days. Proliferation was measured by addition of [^{3}H]thymidine. T-cells from responding cultures were cloned by serial dilution to evaluate their phenotype, function (proliferation, cytokine secretion, and cytolytic activity), mechanism of antigen presentation and cross reactivity with nine structurally related compounds. Lymphocytes were stimulated to proliferate with PIP and PIP-modified serum albumin ([control; 803±250 cpm], [PIP 2 mM; 4554±438 cpm]). Lymphocytes from non-hypersensitive patients were not stimulated ([C; 465.3±310 rpm], [PIP 2 mM; 670.2±489.5 rpm]). CD4+, CD8+, and CD4+CD8+ T-cell clones, which proliferated, killed target cells and secreted high levels of IL4, IL5, IFN-γ, and MIP-1β following PIP or PIP-albumin stimulation, were generated from the hypersensitive patients. The proliferative response was

1. highly specific in terms of drug structure – T-cells were stimulated with PIP, but not structurally-related drugs; and
2. dependent on drug-protein conjugation**.** These data indicate that T cells responsive towards the PIP protein conjugates are present in the circulation of hypersensitive patients.

186 Oxidative status in cystic fibrosis and oral supplementary cysteine

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Oxidative status has a role in the progressive lung damage in CF. Glutathione is an important antioxidant agent and cysteine is the amino-acid that gives to glutathione the antioxidant status. We investigated if oral cysteine has an impact on oxidative status and respiratory function in CF. Oxidative status, estimated on serum d-ROM test and BAP test, and FEV1%pr were performed in 71 pts in which oral whey protein isolate with high content of cysteine (PROther®) was prescribed. All pts showed basal pathological oxidative status (d-ROM mean value: 402 U-CARR, range 223–647 versus normal range 250–300; BAP test mean value 2479 μmol/L, range 1746–3225 versus normal value >2200) and FEV1 < 70% pr. Improvement in oxidative status, even if not significantly, was observed in the 46 pts which completed 6 months of therapy (mean d-ROM test value 391 U-CARR before therapy and 362 after therapy; BAP test value 2621 μmol/L before therapy and 2835 after therapy) and in the 42 pts which completed 12 months of therapy (mean d-ROM test value 365 U-CARR before therapy and 421 after therapy; mean BAP test value 2400 μmol/L before therapy and 2800 after therapy). Beta regression coefficient of FEV1%pr/yr in 66 pts after PROther® supplementation was –0.1 FEV1%pr/yr as mean value (range –12.4, +13.7) and –0.6 FEV1%pr/yr as median value. In 27 pts/66 FEV1%pr improved. Five patients showed no compliance to the treatment. No adverse effect has been reported. Oral addition of cysteine in CF pts is well accepted and tolerated. It seems efficacious to improve oxidative status and, in our data, beta regression coefficient of FEV1%pr/yr is lower than that one normal observed in literature for CF pts.